[Chem. Pharm. Bull. 27(1) 158—165 (1979)]

UDC 547.918.02 : 581.192

Studies on the Chinese Crude Drug "Shoma." IV.[1] Structure of Acetyl Shengmanol Xyloside, a Probable Precursor of Some Known *Cimicifuga* Glycosides[2]

Nobuko Sakurai, Takao Inoue, and Masahiro Nagai

Hoshi College of Pharmacy[3]

(Received August 1, 1978)

Acetyl shengmanol xyloside (I) (monohydrate) isolated from the underground part of *Cimicifuga japonica* (Ranunculaceae) has mp 280—281°, $[\alpha]_D$ −23.7°. The peracetate (II) of I afforded, on acid hydrolysis in aq. methanol, 25-O-methyl cimigenol (VI) along with cimigenol (IV) and isodahurinol (V). I yielded 25-O-methyl cimigenol xyloside (VI) and 25-O-acetyl cimigenol xyloside (VII) on a mild treatment with *p*-toluene sulfonic acid in methanol, while it afforded cimigol 3-xyloside (VIII) on alkali treatment. This transformation of I into VI, and its stereoisomer (VIII) at C-15 and -24 was rationalized in view of different modes of reaction to open the epoxide ring of I on the acidic and the alkaline conditions. Acetyl shengmanol xyloside (I) was degradated with periodate in a solution of cyclohexylamine to afford an aldehyde-carboxylic acid, methyl ester (XIII) of which has structure (XV).

On enzymatic hydrolysis, I yielded its genuine aglycone acetyl shengmanol (XVI), $[\alpha]_D$ −16.1°, as an amorphous powder. The sugar moiety of I was concluded to be attached to C-3 oxygen of the genin (XVI) as β-D-xylopyranose.

On the basis of chemical and spectral data, the structure of acetyl shengmanol xyloside (I) was proposed to be 23*R*,24*S*-3β,15ξ-dihydroxy-23-acetoxy-24,25-epoxy 9,19-cyclolanostan-16-one-3β-D-xylopyranoside. This xyloside (I) is unstable even in the separation procedures from the plant materials, and seems to be a precursor of naturally occurring xylosides of cimigenol, cimigol and their derivatives in *C. japonica*.

Keywords——*Cimicifuga japonica*; Ranunculaceae; acetyl shengmanol xyloside; 9,19-cyclolanostane xyloside; precursor of some *Cimicifuga* xylosides; hydrolytic procedure of glycosides; transformation of acetyl shengmanol xyloside

In preceding paper,[1] it was reported that a triterpene glycoside (I), $C_{37}H_{58}O_{10}\cdot H_2O$, mp 280—281°, $[\alpha]_D$ −23.7° was isolated from the fresh underground part of *Cimicifuga japonica* (Thunb.) Sprengel (Ranunculaceae). Later, the glycoside (I) was designated as acetyl shengmanol xyloside.[2] This report deals with the detail of structure elucidation of the xyloside (I), and also with its facile transformation into some known *Cimicifuga* glycosides.

Acetyl shengmanol xyloside (I) is a monoacetate, since it shows bands at 1735 and 1230 cm^{-1} in its infrared (IR) spectrum, and a three-proton singlet at δ_H 2.05 ppm in its nuculear magnetic resonance (NMR) spectrum. On acetylation with acetic anhydride in pyridine, the xyloside (I) afforded a pentaacetate (II), which showed no hydroxyl absorption in its IR spectrum. Both I and II showed a negative Cotton effect at 316 nm in their circular dichroism (CD) curves, indicating that they have a ketonic function other than acetoxyl carbonyl in the molecules.

The pentaacetate (II) afforded, on hydrolysis with sulfuric acid in aqueous methanol, 25-O-methyl cimigenol (III)[4] as a main aglycone along with cimigenol (IV)[5] and isodahurinol

1) Part III of this series: N. Sakurai, M. Nagai, and T. Inoue, *Yakugaku Zasshi*, **95**, 1354 (1975).
2) A part of this study was briefly reported in *Chem. Pharm. Bull.* (Tokyo), **24**, 3220 (1976), and presented at the 26th IUPAC Congress, Tokyo, September, 1977.
3) Location: *Ebara 2-4-41, Shinagawa-ku, Tokyo.*
4) T. Takemoto and G. Kusano, *Yakugaku Zasshi*, **88**, 623 (1968).
5) T. Takemoto and G. Kusano, *Yakugaku Zasshi*, **87**, 1569 (1967).

(V).[6] Xylose was detected in water soluble fraction of the hydrolysate by paper partition chromatography (PPC). On the other hand, acetyl shengmanol xyloside (I) was treated with *p*-toluene sulfonic acid in methanol at room temperature. The two products of this treatment were directly identified with authentic samples—25-O-methyl cimigenol xyloside (VI)[7] as a main product and 25-O-acetyl cimigenol xyloside (VII)[7] as a minor. These xylosides are well known as ingredients distributed widely in *Cimicifuga* plants. However since the xyloside (I) does not have any O-methyl group as shown in its NMR spectrum, it is apparent that the genuine aglycone of acetyl shengmanol xyloside (I) is not 25-O-methyl cimigenol (III) but some 9, 19-cyclolanostane type triterpenoid that is convertible to III under the acidic conditions.

Alkaline treatment of the xyloside (I) or its peracetate (II) with potassium hydroxide in methanol (or ethanol) or with sodium ethoxide in ethanol, yielded a glycoside (VIII), $C_{35}H_{56}O_9$. Since no carbonyl absorption was observed in IR spectrum of VIII, some structural change of the xyloside (I) in addition to desacetylation must occur during this alkaline treatment. In fact, the glycoside (VIII) never regenerated the pentaacetate (II) on acetylation, but afforded a triacetate (IX) and a tetraacetate (X). The former acetate (IX) could be further acetylated to the latter (X), which still showed a hydroxyl absorption in its IR spectrum.

Table I. ^{1}H-NMR Data (δ value, J in Hz)

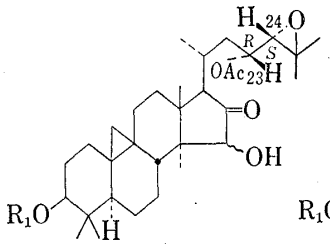
Compounds	3-H	15-H	23-H	24-H	Methyl groups attached at C-25
II	3.12m	5.25 s	~4.9m	2.76 d (J=8.4)	1.31(3H), 1.35(3H)
IX	~3.1m	3.80 d (J=8.0)	~4.4m	3.54 d (J=4.0)	1.21(3H), 1.30(3H)
X	~3.1m	5.00 s	4.42m	3.36 d (J=4.0)	1.19(3H), 1.29(3H)
XI	3.32m	3.81 s	4.46m	3.55 d (J=4.8)	1.22(3H), 1.32(3H)
XIII	3.24m	9.43 s (-CHO)	~3.5m	2.68 d (J=7.2)	1.26(3H), 1.31(3H)
XIV	3.26m	9.46 s (-CHO)	4.83m	2.74 d (J=7.2)	1.29(6H)
XV	4.58m	9.38 s (-CHO)	4.86 (sextet)	2.76 d (J=7.2)	1.31(6H)
XVI	~3.3m	3.98 s	4.93 (sextet)	2.80 d (J=8.4)	1.32(3H), 1.37(3H)

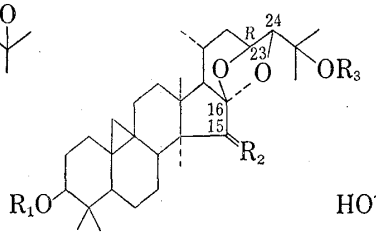
Previously we reported a hydrolytic procedure of glycosides with sodium metaperiodate and cyclohexylamine.[8] This procedure was applied to the hydrolysis of glycoside (VIII), affording cimigol (XI)[9] and its formate (XII). In NMR spectrum of XII, a one-proton singlet at δ_H 8.14 ppm and a one-proton multiplet at δ_H 4.70 ppm are ascribable to a formyloxy proton and the proton on C-3 bearing the formyloxy group, respectively. Signals due to protons at C-23 and C-24 of cimigol (XI) exhibited a good correspondence to those of the genin part of the triacetate (IX), as summarized in Table I. From these evidences, the glycoside (VIII) was concluded to be cimigol 3-xyloside (VIII). By the way, Takemoto and Kusano[9] have reported the isolation of cimigol (XI) from three species of *Cimicifuga* including *C. japonica*, and have predicted the presence of glycosides of XI, but such a glycoside

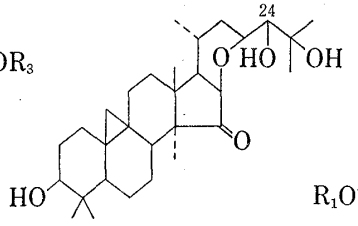
6) G. Kusano, Y. Murakami, N. Sakurai, and T. Takemoto, *Yakugaku Zasshi*, **96**, 82 (1976).
7) T. Takemoto, G. Kusano, and M. Kawahara, *Yakugaku Zasshi*, **90**, 64 (1970).
8) M. Nagai, N. Sakurai, T. Inoue, and K. Kawai, *Yakugaku Zasshi*, **95**, 1350 (1975).
9) G. Kusano and T. Takemoto, *Yakugaku Zasshi*, **95**, 1133 (1975).

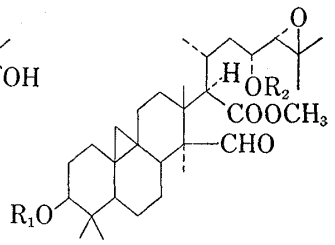
has not been isolated yet in Nature. Cimigol (XI) is reported to be stereoisomeric with cimigenol (IV) at C-15 and C-24 chiral centers.

The above hydrolytic procedure was also applied to hydrolysis of acetyl shengmanol xyloside (I) to give acidic substance. Alkaline treatment[8] and subsequent methylation of the acidic product furnished an aldehyde-methyl ester (XIII), $C_{31}H_{50}O_6$, while alkaline treatment for a shorter period and subsequent methylation of the product yielded a monoacetylated XIII (XIV) as an amorphous powder. Both XIII and XIV afforded, on acetylation. the same diacetate (XV), which no longer showed a hydroxyl absorption in its IR spectrum. The acetyl group of XIV apparently originated from that of acetyl shengmanol xyloside (I), namely, the acetyl moiety of the xyloside (I) is attached to its genin part. The presence of an aldehyde function in XIII was proved from its NMR spectrum—a doublet at δ_C 206.7 ppm as well as a singlet due to an ester carbonyl at δ_C 174.1 ppm. Some chemical shifts of the NMR spectra of pentaacetate (II), aldehyde-methyl ester (XIII), monoacetate (XIV), and diacetate (XV) were summarized also in Table I, as well as those of the genuine aglycone (XVI) of acetyl shengmanol xyloside (I) (*vide infra*). Since the aldehyde proton of XIII, XIV and XV was observed as a singlet, the α-carbon to the aldehyde function has no proton on it. In nuclear magnetic double resonance (NMDR) experiments of the diacetate (XV), a doublet at δ_H 2.76 ppm varied to a singlet on irradiation at δ_H 4.86 ppm, and the sextet at δ_H 4.86 ppm to a double-doublet on irradiation at δ_H 2.76 ppm. The diacetate (XV) ($C_{35}H_{54}O_8$) has eight oxygens in the molecule, seven out of which account for three ester functions and one formyl group in XV. Because XV has no hydroxyl function, the remaining one oxygen has to form an ether linkage. The proton observed as the doublet at δ_H 2.76 ppm is assignable to the one on a carbon bearing the ether oxygen. NMDR experiments disclosed that similarly to the diacetate (XV), the pentaacetate (II) has a proton (24-H) observed at δ_H 2.76 ppm as a doublet coupled with a proton (23-H) at δ_H 4.9 ppm. From







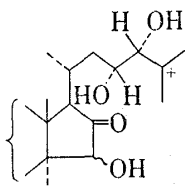


I: R_1=β-D-xyl (p)
XVI: R_1=H

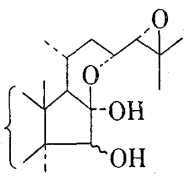
III: R_1=H, R_2=  , R_3=CH_3, 24*S*
IV: R_1=R_3=H, R_2=  , 24*S*
VI: R_1=xyl(p), R_2=  , R_3=CH_3, 24*S*
VII: R_1=xyl(p), R_2=  , R_3=$COCH_3$, 24*S*
VIII: R_1=xyl(p), R_2=  , R_3=H, 24*R*
XI: R_1=R_3=H, R_2=  , 24*R*
XII: R_1=CHO, R_2=  , R_3=H, 24*R*
XVII: R_1=xyl(p), R_2=  , R_3=H, 24*S*

V : 24*S*

XIII: R_1=R_2=H
XIV: R_1=H, R_2=$COCH_3$
XV: R_1=R_2=$COCH_3$



intermediate a



intermediate b

Chart 1

these evidences it was presumed that the genin of acetyl shengmanol xyloside (I) has the same side chain structure as those of pentaacetate (II), monoacetate (XIV) and diacetate (XV).

From all these findings, oxygen atoms of the genin of acetyl shengmanol xyloside (I) are considered to link to C-3, 15, 16, 23, 24, and 25 of 9, 19-cyclolanostane skeleton and the presence of 15-hydroxy-16-oxo- and 23-acetoxy-24,25-epoxy structures is necessary for the partial structures of the genin. The existence of a ketonic function at C-16 of the genin accounts well for the strong negative Cotton effect, $[\theta]_{316}$ -1.86×10^4 in the CD curve of the xyloside (I).[10]

In order to obtain the genuine aglycone of acetyl shengmanol xyloside (I), enzymatic hydrolysis using crude hesperidinase[11] was applied to the hydrolysis of I. The aglycone, acetyl shengmanol (XVI), $[\alpha]_D$ $-16.1°$, was isolated as an amorphous substance, which shows a ketonic (1730 cm^{-1}) and an acetoxy (1738, 1235 cm^{-1}) bands in its IR spectrum, a three proton singlet (acetoxy) at δ_H 2.07 ppm in its NMR spectrum, and a negative Cotton effect, $[\theta]_{316}$ -1.11×10^4 in its CD curve. Alkali treatment of acetyl shengmanol (XVI) afforded cimigol (XI), and periodate oxidation of XVI followed by methylation and acetylation gave diacetate (XV).

The transformation of acetyl shengmanol xyloside (I) into 25-O-methyl cimigenol xyloside (VI) under the acidic condition and into cimigol xyloside (VIII) on the alkaline treatment, could be rationalized as follows. The acid-catalyzed opening of the 24,25-epoxide ring proceeds probably through a carbonium ion intermediate shown as intermediate a in Chart 1, with retention of the configuration at C-24. Addition of methanol (or water) in the reaction solvent to the ionic center at C-25 follows the epoxide ring opening. The resulted alcohol forms ketal derivatives with 24*S* configuration such as 25-O-methyl cimigenol xyloside (VI) under the acidic condition. On the other hand, when I was treated with alkali, desacetylation and subsequent hemiketal formation (intermediate b in Chart 1) between 16-keto group and 23-hydroxyl occur first and then the hemiketal hydroxyl at C-16 attacks C-24 to open the epoxide ring with inversion of its configuration. This reaction mechanism explains the formation of ketal derivatives with 24*R* configuration such as cimigol xyloside (VIII) on the alkaline treatment of the xyloside (I).

The sugar moiety xylose of acetyl shengmanol xyloside (I) was concluded to be attached to the genin acetyl shengmanol (XVI) as β-D-xylopyranose from the following evidences. Methyl α, and β-2, 3, 4-tri-O-methyl-xylopyranoside was detected in methanolysis product of permethylate of 25-O-methyl cimigenol xyloside (VI) obtained oy Hakomori's methylation procedure.[12] Since the pentaacetate (II) showed an anomeric proton as a doublet (J=7.0 Hz) at δ_H 4.50 ppm, the xylose of I is linked in β-orientation, which was also supported by application of Klyne's rule.[13] $[M]_D$ of I minus $[M]_D$ of XVI equals $-72°$; $[M]_D$ (methyl α-D-xylopyranoside) $+253°$, $[M]_D$ (methyl β-D-xylopyranoside) $-108°$.[14]

Based on the above described evidences and discussions we proposed 23*R*, 24*S*-3β, 15ξ-dihydroxy-23-acetoxy-24,25-epoxy-9,19-cyclolanostan-16-one-3-β-D-xylopyranoside for the structure of acetyl shengmanol xyloside (I). It seems noteworthy that acetyl shengmanol xyloside (I) is unstable even in the separation procedures from the plant materials. In fact, we separated I in a poor yield from an ethyl acetate soluble fraction of the methanolic extract of the plant by means of chromatography.[1] But the xyloside (I) seems to constitute more than 10% of the ethyl acetate soluble fraction, since the pentaacetate (II) was obtained in a relatively high yield through a chromatographic separation of the acetylation product of

10) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill, New York, 1960, Chapter IV.
11) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975).
12) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
13) W. Klyne, *Biochem. J.* **47**, XLI (1950).
14) E.L. Eliel, N.L. Allinger, S.J. Angyal, and G.A. Morrison, "Conformational Analysis," Wiley-Interscience, New York, 1965, p. 388.

the same fraction (see Experimental). We consider that acetyl shengmanol xyloside (I) is biosynthetically a precursor of some *Cimicifuga* glycosides such as cimigenol xyloside (XVII),[15] 25-O-methyl cimigenol xyloside (VI) and cimigol xyloside (VIII).

Experimental[16]

Properties of Acetyl Shengmanol Xyloside (I)——Acetyl shengmanol xyloside monohydrate (I) has mp 280—281°, and $[\alpha]_D^{27}$ −23.7° (c=1.1, CH_2Cl_2–MeOH (1:1)). *Anal.* Calcd. for $C_{37}H_{58}O_{10}\cdot H_2O$: C, 65.27; H, 8.88. Found: C, 65.63; H, 8.61. IR ν_{max}^{KBr} cm^{-1}: 3600—3100, 1025 (OH), 1735, 1230 (CO, $OCOCH_3$). NMR (pyridine-d_5) δ ppm: 2.05 ($OCOCH_3$). CD ($c=1.62\times10^{-3}$, MeOH): $[\theta]_{316}$ -1.86×10^4 (negative maximum).

Acetylation of Acetyl Shengmanol Xyloside (I)——Acetyl shengmanol xyloside (I) (100 mg) was acetylated overnight with Ac_2O (1 ml) in pyridine (1 ml) at room temperature. After working up in the usual manner, the product was passed through a silica gel column (1.2 g) (eluent, benzene–EtOAc (85:15)) and crystallized from EtOH to give pentaacetate (II) as colorless needles (10 mg), mp 188—189°, $[\alpha]_D^{20}$ −35.7° (c=1.5, $CHCl_3$). *Anal.* Calcd. for $C_{45}H_{66}O_{14}\cdot H_2O$: C, 63.66; H, 8.07. Found: C, 63.65; H, 7.75. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1740, 1210 ($OCOCH_3$). NMR (pyridine-d_5) δ ppm: 1.95 (3H, s, $OCOCH_3$), 2.02 (6H, s, $2\times OCOCH_3$), 2.10 (3H, s, $OCOCH_3$), 2.17 (3H, s, $OCOCH_3$). NMR ($CDCl_3$) δ ppm: 0.42 (1H, d, J=4.0, cyclopropane methylene), 0.64 (1H, d, J=4.0, cyclopropane methylene), 0.77 (3H, s, *tert.* CH_3), 0.90 (3H, s, *tert.* CH_3), 1.00 (3H, s, *tert.* CH_3), 1.11 (3H, s, *tert.* CH_3), 1.31 (3H, s, *tert.* CH_3), 1.35 (3H, s, *tert.* CH_3), 2.04 (9H, s, $3\times OCOCH_3$), 2.09 (3H, s, $OCOCH_3$), 2.13 (3H, s, $OCOCH_3$), 2.76 (1H, d, J=8.4, 24-H), 3.12 (1H, m, 3-H), 4.50 (1H, d, J=7.0, anomeric H), ~4.9 (1H, m, 23-H), 5.25 (1H, s, 15-H). The doublet at δ 2.76 ppm varied to a singlet on irradiation at δ 4.9 ppm, and the multiplet at δ 4.9 ppm to a double doublet on irradiation at δ 2.76 ppm. MS m/e: 830 (M^+), 815 (M^+-CH_3), 812 (M^+-H_2O), 793 ($M^+-CH_3-H_2O$), 770 (M^+-AcOH), 755 ($M^+-AcOH-CH_3$), 752 ($M^+-AcOH-H_2O$), 710 ($M^+-2AcOH$), 695 ($M^+-2AcOH-CH_3$), 639 ($M^+-2AcOH-$ ). CD ($c=9.25\times10^{-4}$, MeOH): $[\theta]_{316}$ -1.18×10^4 (negative maximum).

Acid Hydrolysis of Pentaacetate (II)——A solution of II (50 mg) in 5% H_2SO_4–50% MeOH (50 ml) was refluxed for 5.5 hr. MeOH was removed *in vacuo*. The resulting solution was extracted with ether. The ether solution was washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The crude products were chromatographed over silica gel (1 g). Elution with benzene–EtOAc (27:2) gave 25-O-methyl cimigenol (III) (5 mg). Elution with benzene–EtOAc (20:5) gave cimigenol (IV) (4 mg). Elution with benzene–EtOAc (2:1) gave isodahurinol (V) (1 mg). III and IV were respectively identified with authentic samples by TLC, mixed mp and IR. V was acetylated with Ac_2O (0.1 ml) in pyridine (0.3 ml) in the usual manner, and the product was identified with an authentic sample of isodahurinol diacetate by TLC (solvents, benzene–EtOAc (10:4); $CHCl_3$–MeOH (200:7)). The water soluble fraction of the above hydrolysis product was treated with Dowex 2-X8 (OH^-) (2 g), and concentrated under reduced pressure. The residue was subjected to PPC (Toyo Filter Paper, No. 50, *n*-BuOH–AcOH–H_2O (6:1:2), and $CHCl_3$–MeOH–pyridine–H_2O (200:40:1:5), coloring with aniline–H_3PO_4), and xylose was solely identified.

***p*-Toluene Sulfonic Acid Treatment of Acetyl Shengmanol Xyloside (I)**——To a solution of I (30 mg) in MeOH (5 ml) was added *p*-toluene sulfonic acid (1 mg) and the total mixture was kept for 2 days at room temperature. After removal of the MeOH, the residue was diluted with water, and extracted with EtOAc. The EtOAc layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue was chromatographed over silica gel (1 g). Elution with benzene–EtOAc (2:1) gave 25-O-acetyl cimigenol xyloside (1 mg) (VII), which was identified with an authentic sample by TLC. Further elution with the same solvent and crystallization from MeOH gave 25-O-methyl cimigenol xyloside (VI) (20 mg), mp 268—270°, which was identified with an authentic sample by mixed mp, TLC and IR.

15) N. Sakurai, T. Inoue, and M. Nagai, *Yakugaku Zasshi*, **92**, 724 (1972).

16) Melting points were determined on a Shimadzu micro melting point determination apparatus and uncorrected. Optical rotations were measured with a JASCO DIP-181 automatic polarimeter in a 1 dm tube. NMR spectra were recorded at 100 MHz with a JEOL FX-100 spectrometer, or at 60 MHz with a Hitachi H-60. Tetramethylsilane was used as the internal standard. Chemical shifts are given on the δ scale (ppm). The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, b=broad. Coupling constants (J values) were given in Hz. CD spectra were measured with a JASCO J-40. Mass spectra (MS) were recorded with a Hitachi RMU-7M, a Hitachi RMS-4, or a JEOL JNS-D300. Gas-liquid partition chromatography (GLC) was run on a Shimadzu GC-4A with a hydrogen flame ionization detector. Columns for chromatography were prepared with silica gel (Kanto Chemical Co. Inc., 100 mesh), and silica gel (Merck, 70—230 mesh). TLC was performed on TLC-plates, silica gel 60 F_{254} precoated (Merck), and the detection was carried out by spraying 10% H_2SO_4 followed by heating.

Alkaline Treatment of Pentaacetate (II)——A solution of II (100 mg) in 0.1% NaOEt–EtOH (6 ml) was allowed to stand overnight at room temperature to give white precipitates, which crystallized from EtOH to afford the glycoside (VIII) as colorless needles (54.4 mg), mp 297—299°, $[\alpha]_D^{19}$ +23.7° (c=0.8, CH_2Cl_2–MeOH (1: 1)). *Anal.* Calcd. for $C_{35}H_{56}O_9$: C, 67.71; H, 9.09. Found: C, 67.71; H, 8.89. IR ν_{max}^{KBr} cm^{-1}: 3600—3100, 1100—1000 (OH). CD: no Cotton effect in the region 250—350 nm.

A solution of II (150 mg) in 2.5% KOH–MeOH (45 ml) or in 2.5% KOH–EtOH (50 ml) was refluxed for 1 hr. The solvent was removed by evaporation and the residue was diluted with water, and extracted with EtOAc. The EtOAc layer was washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated. Recrystallization of the residue from EtOH gave the glycoside (VIII), which was identical with the above glycoside (VIII) by mixed mp and TLC.

Alkaline Treatment of Acetyl Shengmanol Xyloside (I)——A solution of I (2 mg) in 0.1% NaOEt–EtOH (2.0 ml) was allowed to stand overnight at room temperature. The solvent was removed *in vacuo* and the residue was diluted with CH_2Cl_2, washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The product was identified with VIII by TLC.

Acetylation of Glycoside (VIII)——The xyloside (VIII) (35 mg) was acetylated overnight with Ac_2O (0.5 ml) in pyridine (1 ml) at room temperature. After working up in the usual manner, the product was passed through a silica gel column (1 g). Crystallization of the residue eluted with benzene–EtOAc (19: 1) gave tetraacetate (X) as colorless needles (3.7 mg), mp 236—237° (from EtOH), $[\alpha]_D^{23}$ +23.3° (c=0.2, $CHCl_3$–MeOH (1: 1)). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3550, 1020 (OH), 1755, 1215 ($OCOCH_3$). NMR ($CDCl_3$) δ ppm: 0.40 (1H, d, J=4.0, cyclopropane methylene), 0.62 (1H, d, J=4.0, cyclopropane methylene), 0.79 (3H, s, *tert.* CH_3), 0.91 (3H, s, *tert.* CH_3), 1.11 (3H, s, *tert.* CH_3), 1.17 (3H, s, *tert.* CH_3), 1.19 (3H, s, *tert.* CH_3), 1.29 (3H, s, *tert.* CH_3), 2.07 (12H, s, 4×$OCOCH_3$), ~3.1 (1H, m, 3-H), 3.36 (1H, d, J=4.0, 24-H), 4.42 (1H, m, 23-H), 4.51 (1H, d, J=7.0, anomeric H), 5.00 (1H, s, 15-H). The multiplet at δ 4.42 ppm varied to a quartet on irradiation at δ 3.36 ppm, and the doublet at δ 3.36 ppm to a singlet on irradiation at δ 4.42 ppm. High resolution mass spectrum (High MS) m/e: Calcd. for $C_{43}H_{64}O_{13}$ (M^+) 788.435, $C_{42}H_{61}O_{13}$ (M^+-CH_3) 773.411, $C_{42}H_{59}O_{12}$ ($M^+-CH_3-H_2O$) 755.400, $C_{41}H_{58}O_{10}$ ($M^+-AcOH-H_2O$) 710.403. Found: 788.435, 773.410, 755.400, 710.408, respectively.

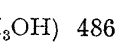
Elution of the column with benzene–EtOAc (9: 1) afforded triacetate (IX) as colorless needles (2 mg), mp 257—258° (from EtOH), $[\alpha]_D^{23}$ +7.1° (c=0.4, $CHCl_3$–MeOH (1: 1)). *Anal.* Calcd. for $C_{41}H_{62}O_{12}$: C, 65.93; H, 8.37. Found: C, 65.69; H, 8.12. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600—3200 (OH), 1750, 1255 ($OCOCH_3$). NMR ($CDCl_3$) δ ppm: 0.36 (1H, d, J=4.2, cyclopropane methylene), 0.60 (1H, d, J=4.2, cyclopropane methylene), 0.78 (3H, s, *tert.* CH_3), 0.91 (3H, s, *tert.* CH_3), 0.92 (3H, s, *tert.* CH_3), 1.08 (3H, s, *tert.* CH_3), 1.21 (3H, s, *tert.* CH_3), 1.30 (3H, s, *tert.* CH_3), 2.04 (9H, s, 3×$OCOCH_3$), 2.64 (1H, d, J=8.0, 15-OH), ~3.1 (1H, m, 3-H), 3.54 (1H, d, J=4.0, 24-H), 3.80 (1H, d, J=8.0, 15-H), ~4.4 (1H, m, 23-H), 4.52 (1H, d, J=6.6, anomeric H). On addition of D_2O the doublet at δ 2.64 ppm disappeared, and the doublet at δ 3.80 ppm varied to a singlet. The doublet at δ 3.54 ppm varied to a singlet on irradiation at δ 4.4 ppm, and the multiplet at δ 4.4 ppm to a doublet-like signal on irradiation at δ 3.54 ppm. Acetylation of IX (2 mg) with Ac_2O (0.2 ml) in pyridine (0.5 ml) yielded the tetraacetate (X).

Hydrolysis of Glycoside (VIII) with Periodate and Cyclohexylamine——To a mixture of VIII (60 mg) dissolved in MeOH (120 ml) and the cyclohexylamine solution (90 ml)[17] was added the $NaIO_4$ solution (24 ml)[18] in equal portions at 0, 13, 15 and 23 hr after the reaction started. The reaction mixture was stirred for 40 hr at room temperature. After evaporation of the solvent (at room temperature), H_2O was added to the residue. The mixture was extracted with ether, and the ether layer was washed with 0.1 N H_2SO_4 and then with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The product was chromatographed over silica gel (1 g). Fraction eluted with benzene–EtOAc (19: 1) afforded cimigol formate (XII) (5 mg), mp 227—228° (from MeOH), $[\alpha]_D^{21}$ +51.9° (c=0.3, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{48}O_6$: C, 72.06; H, 9.36. Found: C, 71.89; H, 8.96. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1728, 1180 (OCHO). NMR ($CDCl_3$) δ ppm: 2.64 (1H, d, J=8.0, 15-OH), 3.46 (1H, d, J=4.8, 24-H), 3.83 (1H, d, J=8.0, 15-H), 4.44 (1H, sextet, 23-H), 4.70 (1H, m, 3-H), 8.14 (1H, s, OCHO). On addition of D_2O the doublet at δ 2.64 ppm disappeared, and the doublet at δ 3.83 ppm varied to a singlet. High MS m/e: Calcd. for $C_{31}H_{48}O_6$ (M^+) 516.345. Found: 516.345.

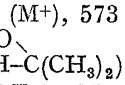
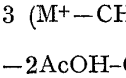
A solution of XII (2 mg) in 2.5% KOH–EtOH (0.5 ml) was kept for 2 hr at room temperature. The reaction mixture was poured into water and extracted with ether. The ether layer was washed with 0.1 N H_2SO_4 and then with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The product was identified with an authentic sample of cimigol (XI) by TLC.

Fraction eluted with benzene–EtOAc (9: 1) afforded cimigol (XI) as colorless needles (3 mg), mp 280—282° (from benzene), $[\alpha]_D^{22}$ +45.7° (c=0.3, EtOH–$CHCl_3$ (7: 2)).[19] This compound (XI) was identified with an authentic sample by mixed mp, IR and TLC.

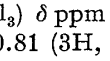
17) Cyclohexylamine solution: Cyclohexylamine (3.0 ml) was mixed with H_2O to make up 1000 ml, and the pH was adjusted to be 8.0 by addition of AcOH~(1.4 ml) to the mixture.
18) Sodium metaperiodate solution: $NaIO_4$ (2.39 g) was dissolved in H_2O to make up 100 ml.
19) The optical rotation of this cimigol was reported erroneously in the previous paper (ref. 2).

Hydrolysis of Acetyl Shengmanol Xyloside (I) with Periodate and Cyclohexylamine——a) To a mixture of I (45 mg) dissolved in MeOH (90 ml) and the cyclohexylamine solution (68 ml) was added the $NaIO_4$ solution (18 ml) in equal portions at 0, 2, 20 and 31 hr after the reaction started. The reaction mixture was stirred for 40 hr at room temperature. After evaporation of the solvent (at room temperature), H_2O was added to the residue. The mixture was extracted with ether, and the ether layer was washed with 0.1 N H_2SO_4 and then with H_2O, dried over anhydrous Na_2SO_4 and concentrated. The product in MeOH (1 ml) was kept overnight at room temperature after addition of 10% Na_2CO_3 (1 ml). The MeOH was removed, and the residue was added with water and washed with ether. The water layer was acidified with 0.1 N H_2SO_4 and extracted with ether. The ether solution was washed with water, dried over anhydrous Na_2SO_4, concentrated, and methylated with CH_2N_2 in ether. The solvent was removed. Recrystallization of the residue from hexane–benzene (1: 1) gave an aldehyde-methyl ester (XIII) (5 mg), mp 161—163°, $[\alpha]_D^{29}$ +44.8° (c=0.9, CH_2Cl_2–MeOH (1: 1)). IR ν_{max}^{KBr} cm^{-1}: 3600—3200, 1050 (OH), 1725, 1162 ($COOCH_3$). NMR ($CDCl_3$) δ ppm: 0.20 (1H, d, J=4.8, cyclopropane methylene), 0.74 (1H, d, J=4.8, cyclopropane methylene), 0.80 (3H, s, *tert.* CH_3), 0.91 (3H, s, *tert.* CH_3), 1.03 (3H, s, *tert.* CH_3), 1.26 (3H, s, *tert.* CH_3), 1.31 (3H, s, *tert.* CH_3), 1.65 (3H, s, *tert.* CH_3), 2.68 (1H, d, J=7.2, 24-H), 3.24 (1H, m, 3-H), ~3.5 (1H, m, 23-H), 3.54 (3H, s, $COOCH_3$), 9.43 (1H, s, CHO). ^{13}C-NMR ($CDCl_3$) δ ppm: 57.2 (q, $COO\underline{C}H_3$), 59.5 (s, C-15), 68.2 (d, C-23 or C-24), 68.9 (d, C-23 or C-24), 78.6 (d, C-3), 174.1 (s, $\underline{C}OOCH_3$), 206.7 (d, CHO). High MS m/e: Calcd. for $C_{31}H_{50}O_6$ (M^+) 518.360, $C_{30}H_{46}O_5$ (M^+-CH_3OH) 486.334, $C_{27}H_{43}O_5$ (M^+- ) 447.310. Found: 518.356, 486.331, 447.305, respectively.

b) I (170 mg) was hydrolyzed with the periodate solution (168 ml) and the cyclohexylamine solution (255 ml) in the same manner. The resulting product in MeOH (1 ml), after addition of 10% Na_2CO_3 (1 ml), was kept for 3 hr at room temperature. After evaporation of MeOH, the hydrolysate was diluted with water and washed with ether. The water layer was acidified with 0.1 N H_2SO_4 and extracted with ether. The ether solution was washed with water, and dried over anhydrous Na_2SO_4. After removal of the solvent the residue was chromatographed over silica gel (1 g). Elution with benzene–EtOAc (17: 3) gave an aldehyde-methyl ester monoacetate (XIV) (20 mg) as an amorphous powder. NMR ($CDCl_3$) δ ppm: 0.21 (1H, d, J=4.8, cyclopropane methylene), 0.78 (3H, s, *tert.* CH_3), 0.89 (3H, s, *tert.* CH_3), 1.01 (3H, s, *tert.* CH_3), 1.29 (6H, s, 2×*tert.* CH_3), 1.58 (3H, s, *tert.* CH_3), 2.08 (3H, s, $OCOCH_3$), 2.74 (1H, d, J=7.2, 24-H), 3.26 (1H, m, 3-H), 3.54 (3H, s, $COOCH_3$), 4.83 (1H, m, 23-H), 9.46 (1H, s, CHO).

Acetylation of Aldehyde-methyl Ester (XIII)——The aldehyde-methyl ester (XIII) was acetylated with Ac_2O (0.6 ml) in pyridine (3 ml) overnight at room temperature. After working up in the usual manner, the product was passed through a silica gel column (0.5 g) (eluent, benzene–EtOAc (19: 1)). After crystallization from MeOH aldehyde-methyl ester diacetate (XV) was obtained as colorless needles (39 mg), mp 176—177°, $[\alpha]_D^{34}$ +24.0° (c=0.8, $CHCl_3$). *Anal.* Calcd. for $C_{35}H_{54}O_8$: C, 69.74; H, 9.03. Found: C, 69.61; H, 8.69. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1732, 1720, 1230, 1165 ($OCOCH_3$, CHO, $COOCH_3$). NMR ($CDCl_3$) δ ppm: 0.21 (1H, d, J=4.2, cyclopropane methylene), 0.79 (3H, s, *tert.* CH_3), 0.89 (3H, s, *tert.* CH_3), 1.03 (3H, s, *tert.* CH_3), 1.31 (6H, s, 2×*tert.* CH_3), 1.59 (3H, s, *tert.* CH_3), 2.05 (3H, s, $OCOCH_3$), 2.09 (3H, s, $OCOCH_3$), 2.76 (1H, d, J=7.2, 24-H), 3.58 (3H, s, $COOCH_3$), 4.58 (1H, m, 3-H), 4.86 (1H, sextet, $J_1=J_2$=12.0, J_3=4.0, 23-H), 9.38 (1H, s, 15-H). MS m/e: 602 (M^+), 573 (M^+-CHO), 542 (M^+-AcOH), 513 (M^+-CHO–AcOH), 482 (M^+-2AcOH), 471 (M^+-AcOH– ), 453 (M^+-CHO–2AcOH), 411 (M^+-2AcOH– ).

Acetylation of Aldehyde-methyl Ester Monoacetate (XIV)——XIV (3 mg) was acetylated overnight with Ac_2O (0.03 ml) in pyridine (0.1 ml) at room temperature. After working up in the usual manner, the product was recrystallized from MeOH and identified with the aldehyde-methyl ester diacetate (XV) by mixed mp, TLC and IR.

Enzymatic Hydrolysis of Acetyl Shengmanol Xyloside (I)——A solution of acetyl shengmanol xyloside (I) (70 mg) in a mixture of EtOH (35 ml) and 1/5 M Na_2HPO_4–1/10 M citric acid buffer (pH 4.0) (70 ml) was treated with crude hesperidinase (70 mg, Tanabe Pharm. Co., Lot. No. 71) in H_2O (35 ml) and the total mixture was kept for 40 hr with gentle stirring at 37°. The incubation mixture was concentrated, and extracted with ether. The ether layer was washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated. The residue was chromatographed over silica gel (1 g). Elution with benzene–EtOAc (17: 3) gave acetyl shengmanol (XVI) as an amorphous powder, $[\alpha]_D^{17}$ −16.1° (c=0.8, $CHCl_3$), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3600—3400 (OH), 1738, 1730, 1235 ($OCOCH_3$, CO). NMR ($CDCl_3$) δ ppm: 0.43 (1H, d, J=4.2, cyclopropane methylene), 0.66 (1H, d, J=4.2, cyclopropane methylene), 0.81 (3H, s, *tert.* CH_3), 0.86 (6H, s, 2×*tert.* CH_3), 0.98 (3H, s, *tert.* CH_3), 1.32 (3H, s, *tert.* CH_3), 1.37 (3H, s, *tert.* CH_3), 2.09 (3H, s, $OCOCH_3$), 2.80 (1H, d, J=8.4, 24-H), ~3.3 (1H, m, 3-H), 3.98 (1H, s, 15-H), 4.93 (1H, sextet, $J_1=J_2$=12.0, J_3=4.0, 23-H). The doublet at δ 2.80 ppm varied to a singlet on irradiation at δ 4.93 ppm, and the multiplet at δ 4.93 ppm to a quartet on irradiation at δ 2.80 ppm. MS m/e: 530 (M^+), 512 (M^+-H_2O). High MS m/e: Calcd. for $C_{32}H_{48}O_5$ (M^+-H_2O) 512.349, $C_{30}H_{44}O_3$ (M^+-AcOH–H_2O) 452.329, $C_{26}H_{39}O_3$ (M^+-AcOH– ) 399.290. Found: 512.347, 452.331, 399.289. CD ($c=6.37\times10^{-4}$, MeOH): $[\theta]_{316}$ -1.11×10^4 (negative maximum).

A solution of XVI (1 mg) in 2.5% KOH–EtOH (0.5 ml) was heated at 70° for 1 hr. The reaction mixture was diluted with water, and extracted with ether. The ether layer was washed with 0.1 N H_2SO_4 and then

with H_2O, dried over anhydrous Na_2SO_4 and concentrated to give cimigol (XI), which was identified with an authentic sample by TLC.

A solution of XVI (11.1 mg) in a mixture of MeOH (5 ml), H_2O (4 ml) and 0.1 N $NaIO_4$ (2 ml) was stirred for 3 hr at room temperature. After evaporation of the solvent at room temperature, the residue was diluted with water, alkalified with 10% Na_2CO_3 and washed with ether. The water layer was acidified with 0.1 N H_2SO_4, and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4, concentrated and methylated with CH_2N_2 in ether. After the solvent was removed, the product was acetylated overnight with Ac_2O (0.1 ml) in pyridine (1 ml) at room temperature. After working-up in the usual way the product was chromatographed over silica gel (500 mg). Elution with benzene–EtOAc (19: 1) afforded the aldehyde-methyl ester diacetate (XV) (3.6 mg) as colorless needles, mp 176—177° (from MeOH), which were identified with an authentic sample of XV by mixed mp, TLC and IR.

Methylation followed by Methanolysis of 25-O-Methyl Cimigenol Xyloside (VI)——i) Dimsyl carbanion: A mixture of NaH (25 mg) and DMSO (1 ml) was heated under a N_2 atmosphere at 70—80° for 30 min to yield slightly greenish dimsyl carbanion.

ii) To a solution of 25-O-methyl cimigenol xyloside (VI) (5 mg) in DMSO (1 ml) was added dimsyl carbanion (prepared as above) and the total mixture was kept under stirring for 50 min at room temperature, treated with CH_3I (1 ml), kept under stirring for 1 hr, and poured into water. The product was taken up in EtOAc, washed with water, dried over anhydrous Na_2SO_4, and concentrated. The product was chromatographed over silica gel (0.5 g). Elution with benzene–EtOAc (19: 1) gave permethylate. A solution of permethylate in 10% HCl–MeOH (1 ml) was refluxed for 40 min. The MeOH was removed, and the residue was diluted with water and extracted with $CHCl_3$. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The residue was shown to contain methyl α- and β-2,3,4-tri-O-methyl xylopyranoside by TLC (benzene–acetone) (6: 1)) and GLC: 1.5% OV-17 on Shimalite W (80—100 mesh), 4 mm × 1.5 m, column temp. 102°, carrier gas N_2 (1 kg/cm^2), t_R (min): 5.15, 6.95. t_R of standard sample (min): 5.18, 6.95.

Direct Isolation of Pentaacetate (II) from a Plant Extract——An EtOAc soluble fraction (12.7 g) of the MeOH extract of *C. japonica* was acetylated with Ac_2O (15 ml) in pyridine (30 ml) overnight at room temperature. The solvent was removed *in vacuo*. After working up in the usual manner, the product (15.8 g) was passed through a silica gel column (500 g) (eluent, benzene–EtOAc (85: 15)) to give pentaacetate (II) (3.18 g) as a syrupy oil. Crystallization from EtOH furnished pentaacetate (II) (1.8 g), mp 188—189°, which was identified with acetylation product (II) of acetyl shengmanol xyloside (I) by TLC, IR and mixed mp.

Acknowledgement The authors are grateful to prof. T. Takemoto, Tokushima Bunri University and Dr. G. Kusano, Tohoku University for a gift of cimigol. They are also indebted to Dr. N. Takamura, Tanabe Pharm. Co., for a generous gift of crude hesperidinase and Mr. Higashiyama of this College for measurement of NMR and MS spectra.